US009207239B2

(12) **United States Patent**
Kasdan et al.

(10) **Patent No.: US 9,207,239 B2**
(45) **Date of Patent: *Dec. 8, 2015**

(54) **KITS, COMPOSITIONS AND METHODS FOR DETECTING A BIOLOGICAL CONDITION**

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(73) Assignee: **LEUKODX LTD.**, Jerusalem (IL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **14/571,906**

(22) Filed: **Dec. 16, 2014**

(65) **Prior Publication Data**

US 2015/0132776 A1 May 14, 2015

Related U.S. Application Data

(60) Continuation of application No. 14/296,317, filed on Jun. 4, 2014, now Pat. No. 8,945,913, which is a division of application No. 13/716,246, filed on Dec. 17, 2012, now abandoned.

(51) **Int. Cl.**

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G01N 33/68	(2006.01)
B01L 3/00	(2006.01)

(52) **U.S. Cl.**

CPC ***G01N 33/56972*** (2013.01); ***B01L 3/502*** (2013.01); ***B01L 3/5027*** (2013.01);

(Continued)

(58) **Field of Classification Search**

CPC . B01L 3/502; B01L 3/5027; B01L 3/502715; G01N 33/56972; G01N 33/68; G01N 33/569

See application file for complete search history.

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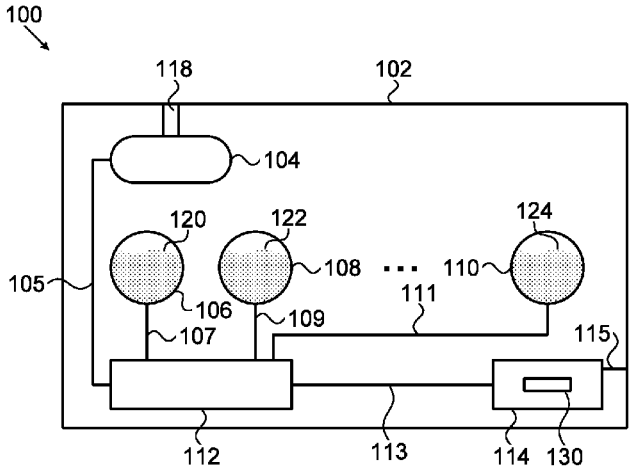
Primary Examiner — Melanie Y Brown

(74) *Attorney, Agent, or Firm* — Wilson Sonsini Goodrich & Rosati

(57) **ABSTRACT**

The present invention provides kits, apparatus and methods for determining a biological condition in a mammalian subject, the method includes incubating a specimen from a patient with at least one composition in a kit for a predetermined period of time to form at least one reaction product, when the subject has said biological condition, and receiving an indication of the at least one reaction product responsive to at least one reporter element in the kit thereby providing the indication of the biological condition in the subject.

27 Claims, 11 Drawing Sheets



(52) **U.S. Cl.**
CPC ***B01L3/502715*** (2013.01); ***G01N 33/569*** (2013.01); ***G01N 33/68*** (2013.01); *B01L 2200/10* (2013.01); *B01L 2300/0816* (2013.01); *B01L 2300/0867* (2013.01); *B01L 2300/0883* (2013.01); *B01L 2400/0481* (2013.01); *G01N 2333/70535* (2013.01); *G01N 2333/70596* (2013.01)

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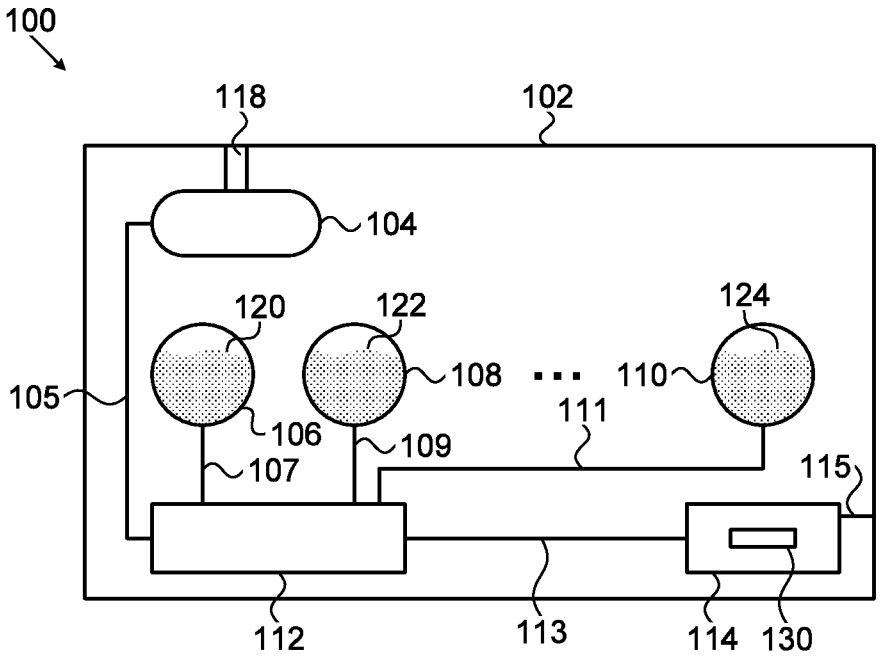
100
118
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FIG. 1

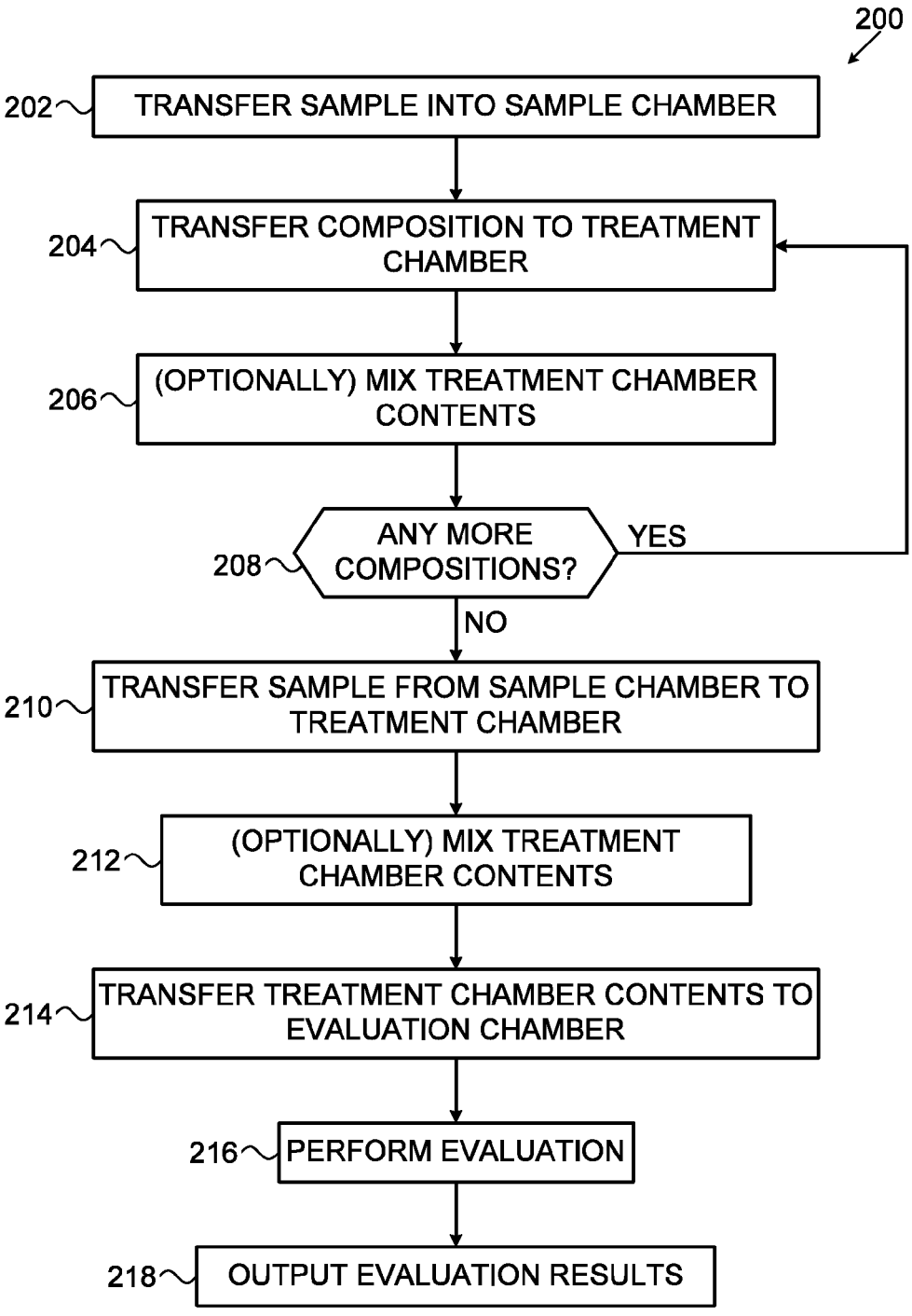

200
202
TRANSFER SAMPLE INTO SAMPLE CHAMBER
204
TRANSFER COMPOSITION TO TREATMENT CHAMBER
206
(OPTIONALLY) MIX TREATMENT CHAMBER CONTENTS
208
ANY MORE COMPOSITIONS?
YES
NO
210
TRANSFER SAMPLE FROM SAMPLE CHAMBER TO TREATMENT CHAMBER
212
(OPTIONALLY) MIX TREATMENT CHAMBER CONTENTS
214
TRANSFER TREATMENT CHAMBER CONTENTS TO EVALUATION CHAMBER
216
PERFORM EVALUATION
218
OUTPUT EVALUATION RESULTS

FIG. 2

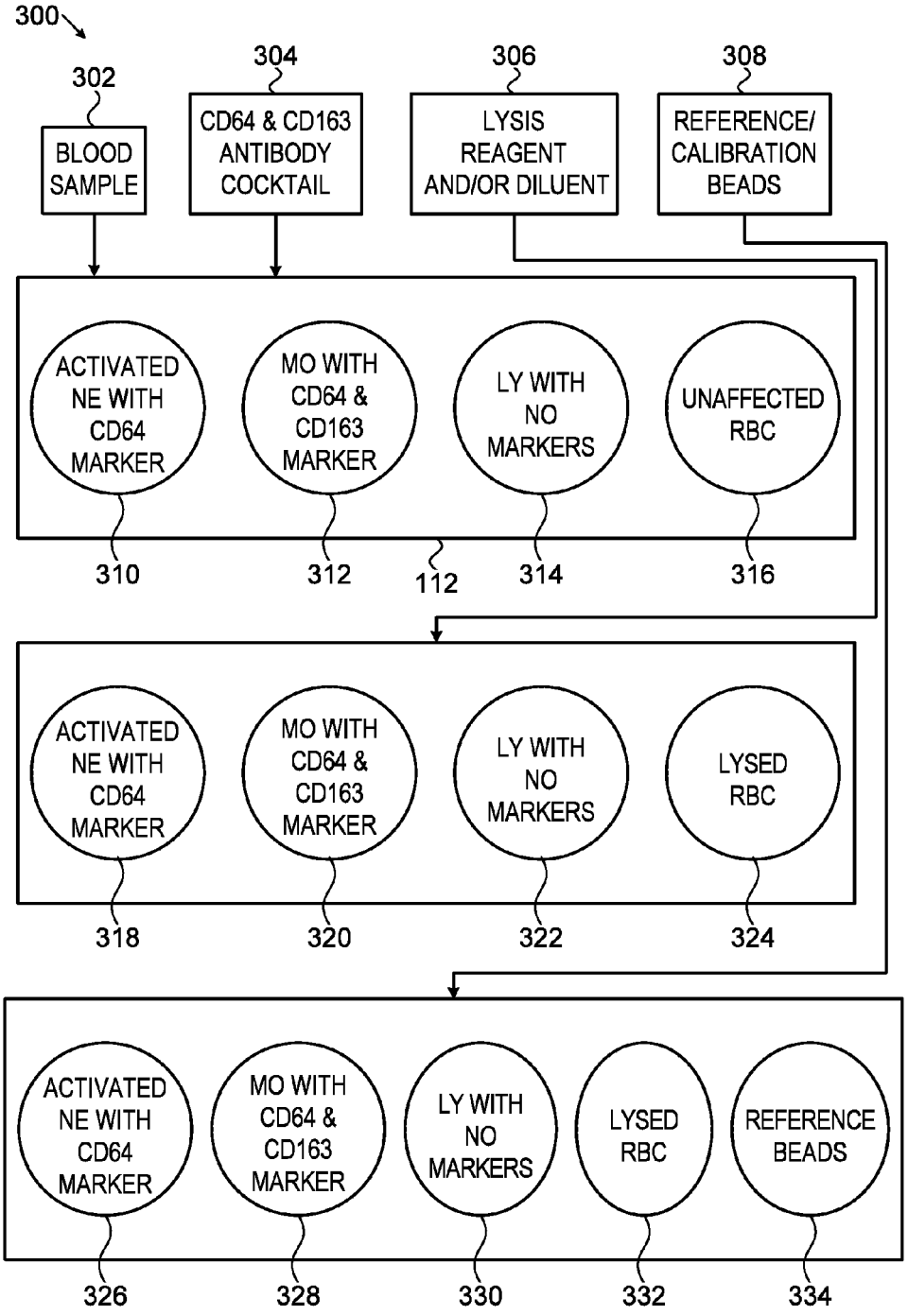

300
302
BLOOD SAMPLE
304
CD64 & CD163 ANTIBODY COCKTAIL
306
LYSIS REAGENT AND/OR DILUENT
308
REFERENCE/ CALIBRATION BEADS
ACTIVATED NE WITH CD64 MARKER
MO WITH CD64 & CD163 MARKER
LY WITH NO MARKERS
UNAFFECTED RBC
310
312
112
314
316
ACTIVATED NE WITH CD64 MARKER
MO WITH CD64 & CD163 MARKER
LY WITH NO MARKERS
LYSED RBC
318
320
322
324
ACTIVATED NE WITH CD64 MARKER
MO WITH CD64 & CD163 MARKER
LY WITH NO MARKERS
LYSED RBC
REFERENCE BEADS
326
328
330
332
334

FIG. 3

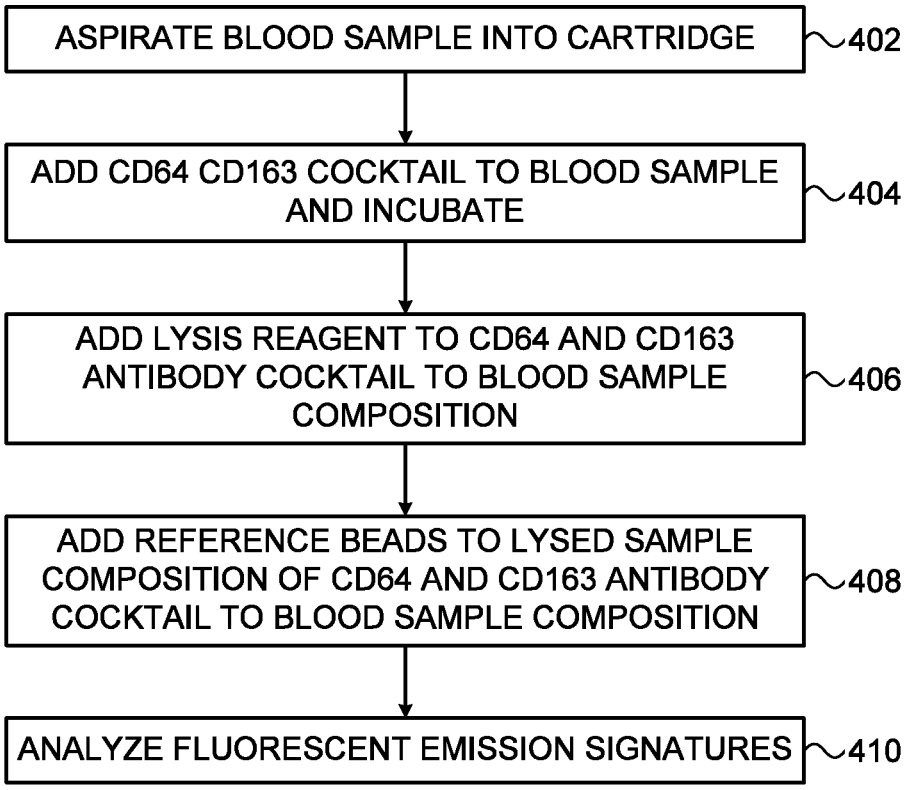

ASPIRATE BLOOD SAMPLE INTO CARTRIDGE
402
ADD CD64 CD163 COCKTAIL TO BLOOD SAMPLE AND INCUBATE
404
ADD LYSIS REAGENT TO CD64 AND CD163 ANTIBODY COCKTAIL TO BLOOD SAMPLE COMPOSITION
406
ADD REFERENCE BEADS TO LYSED SAMPLE COMPOSITION OF CD64 AND CD163 ANTIBODY COCKTAIL TO BLOOD SAMPLE COMPOSITION
408
ANALYZE FLUORESCENT EMISSION SIGNATURES
410

FIG. 4

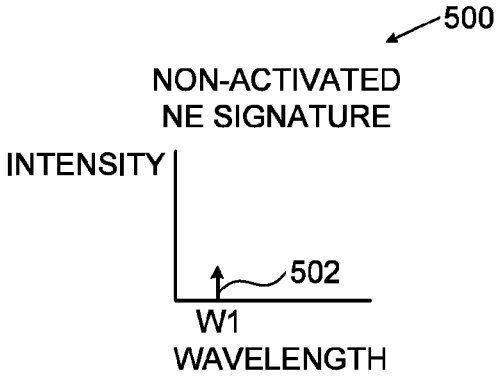
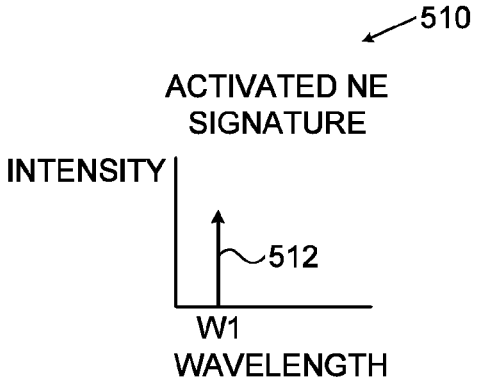


FIG. 5A



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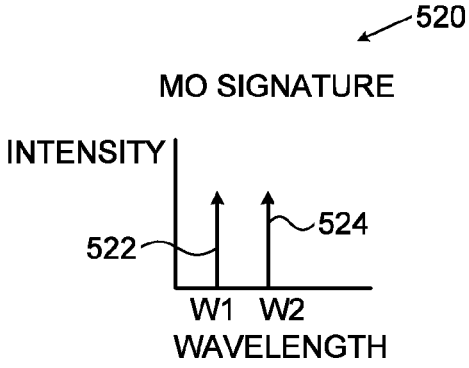


FIG. 5C

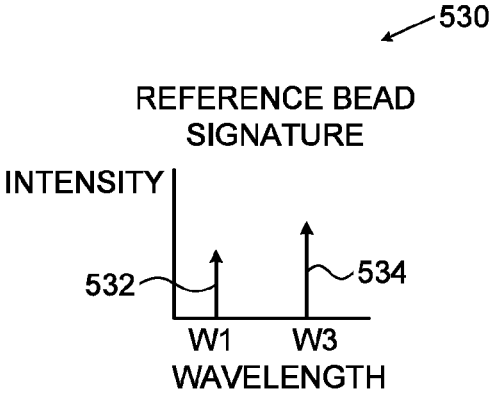


FIG. 5D

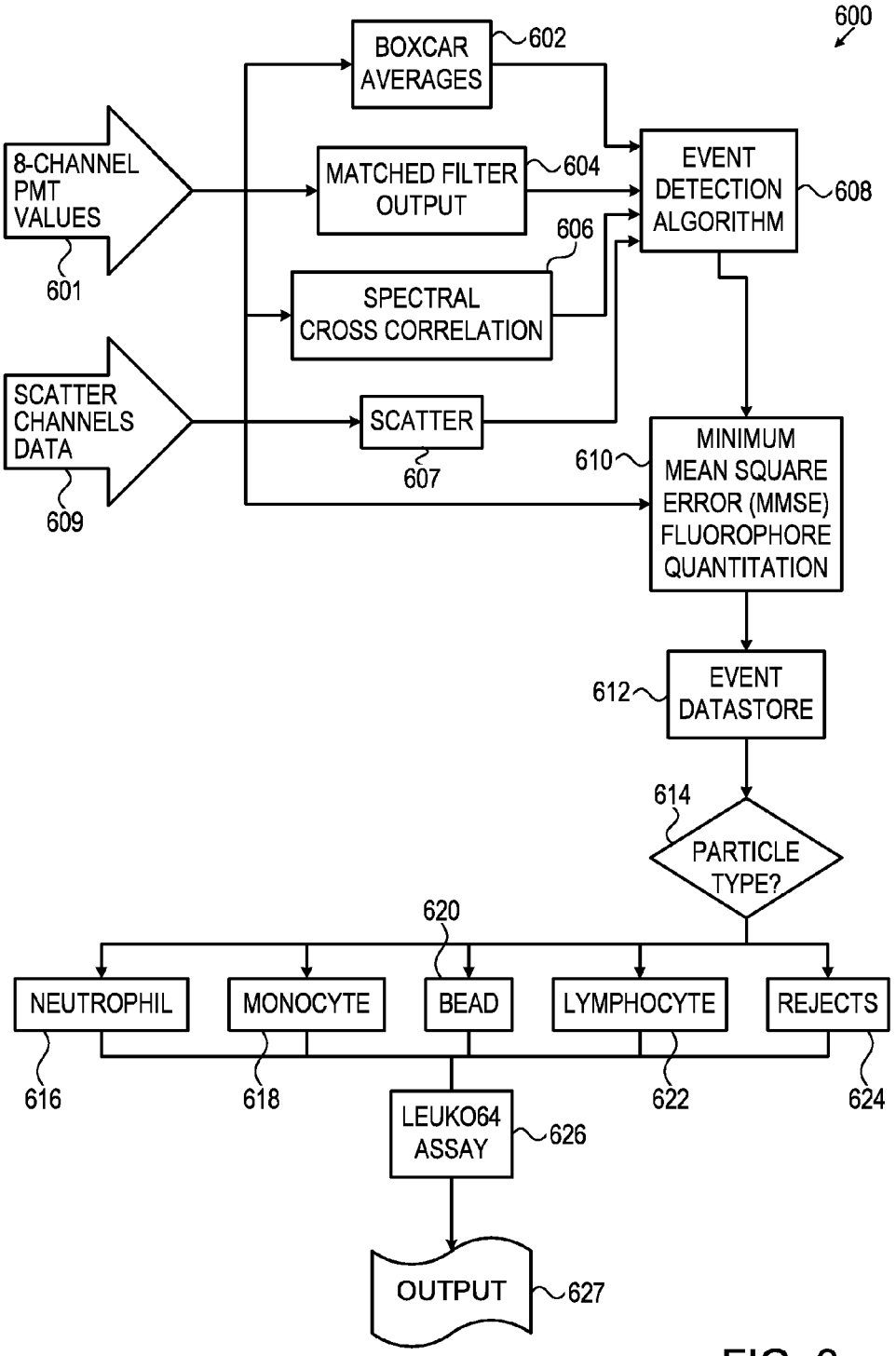
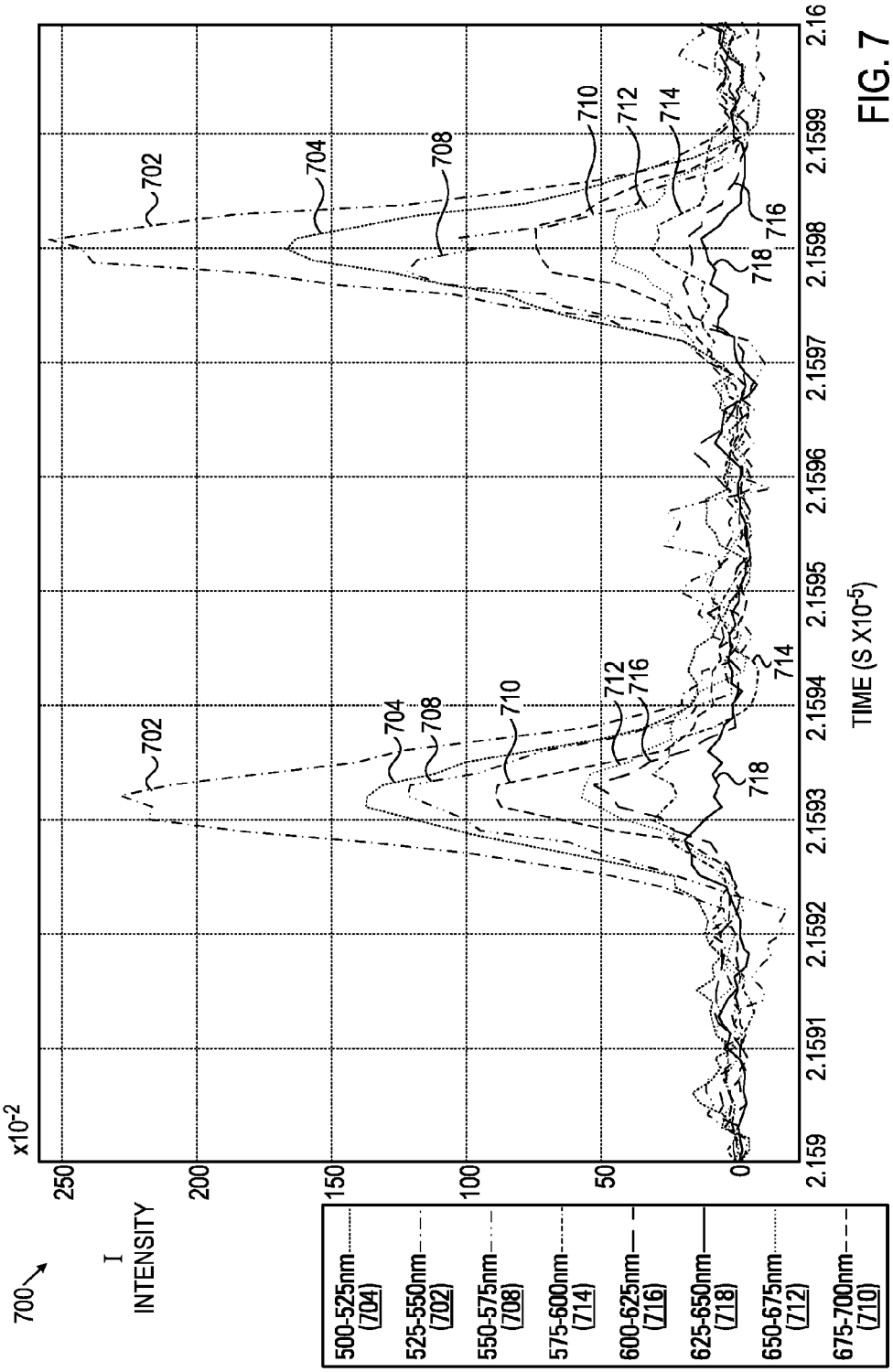

600
BOXCAR AVERAGES
602
8-CHANNEL PMT VALUES
601
MATCHED FILTER OUTPUT
604
EVENT DETECTION ALGORITHM
608
606
SPECTRAL CROSS CORRELATION
SCATTER CHANNELS DATA
609
SCATTER
607
610
MINIMUM MEAN SQUARE ERROR (MMSE) FLUOROPHORE QUANTITATION
612
EVENT DATASTORE
614
PARTICLE TYPE?
620
NEUTROPHIL
MONOCYTE
BEAD
LYMPHOCYTE
REJECTS
616
618
622
624
LEUKO64 ASSAY
626
OUTPUT
627

FIG. 6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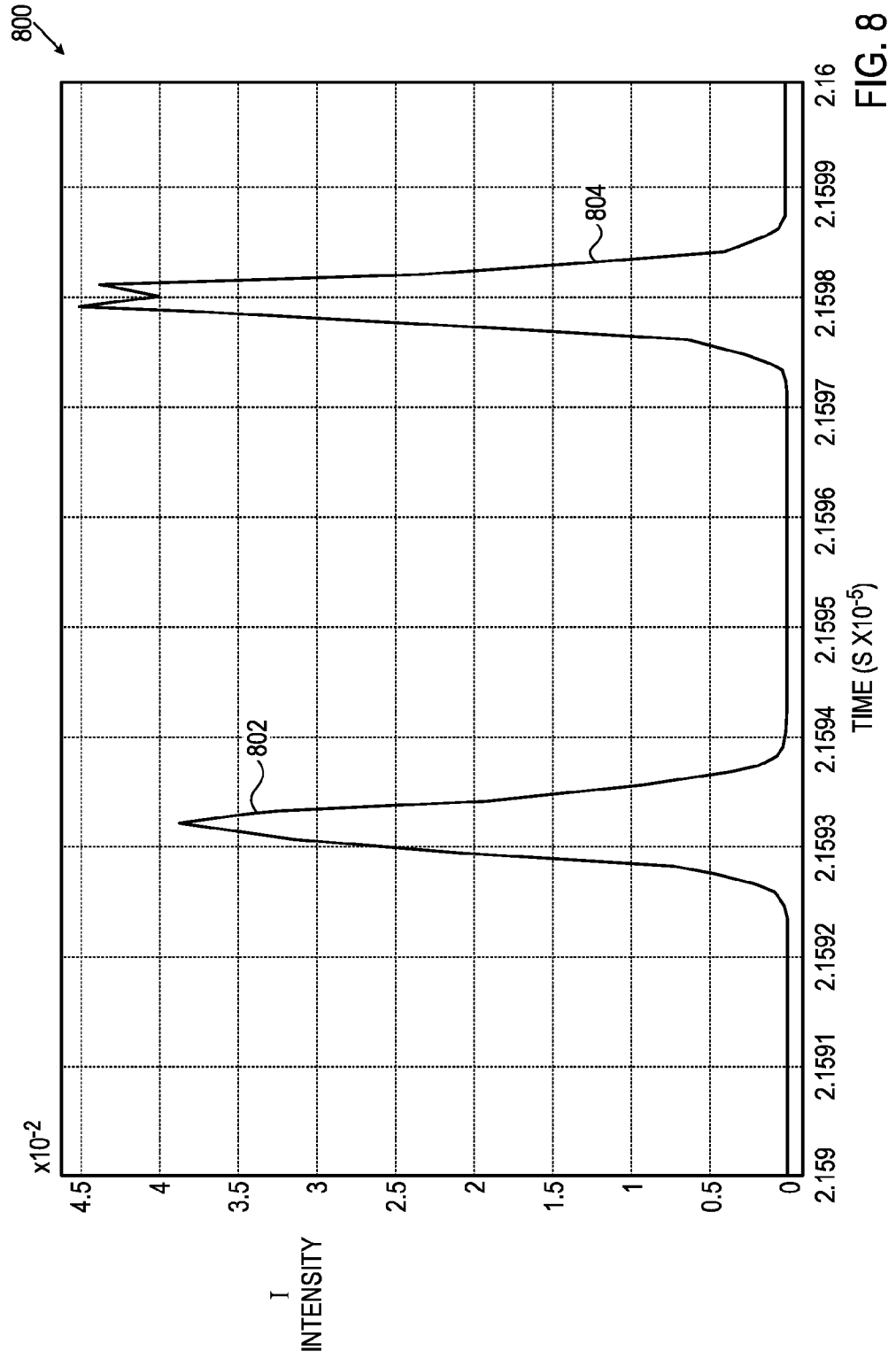
I
INTENSITY
x10-2
250
200
150
100
50
0
2.159
2.1591
2.1592
2.1593
2.1594
2.1595
2.1596
2.1597
2.1598
2.1599
2.16
TIME (S X10-5)
702
704
708
710
712
714
716
718
500-525nm (704)
525-550nm (702)
550-575nm (708)
575-600nm (714)
600-625nm (716)
625-650nm (718)
650-675nm (712)
675-700nm (710)

FIG. 7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804
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4
3.5
3
2.5
2
1.5
1
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I
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2.1591
2.1592
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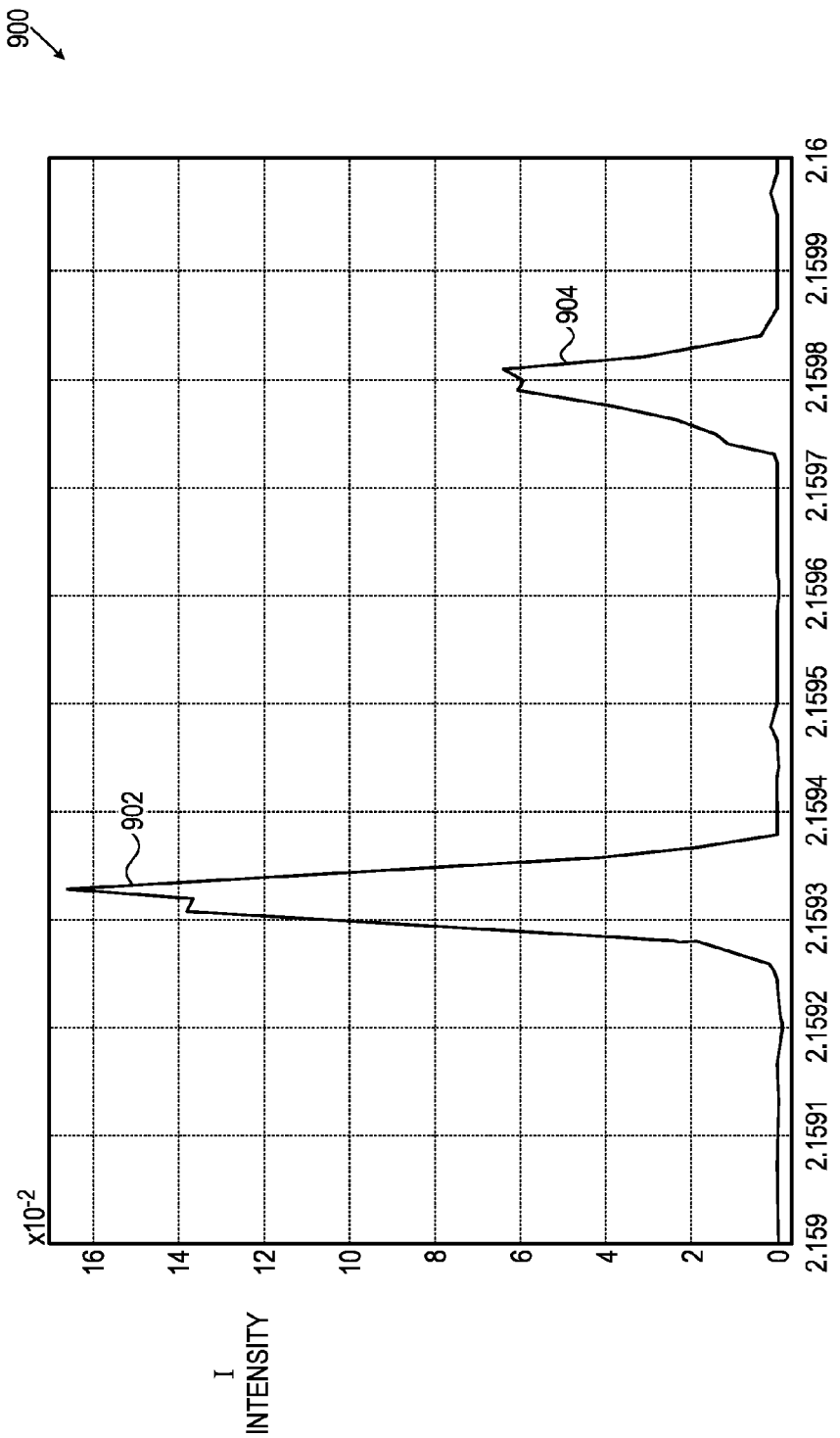
2.1598
2.1599
2.16
TIME (S X10-5)

FIG. 8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14
12
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6
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2.159
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2.1593
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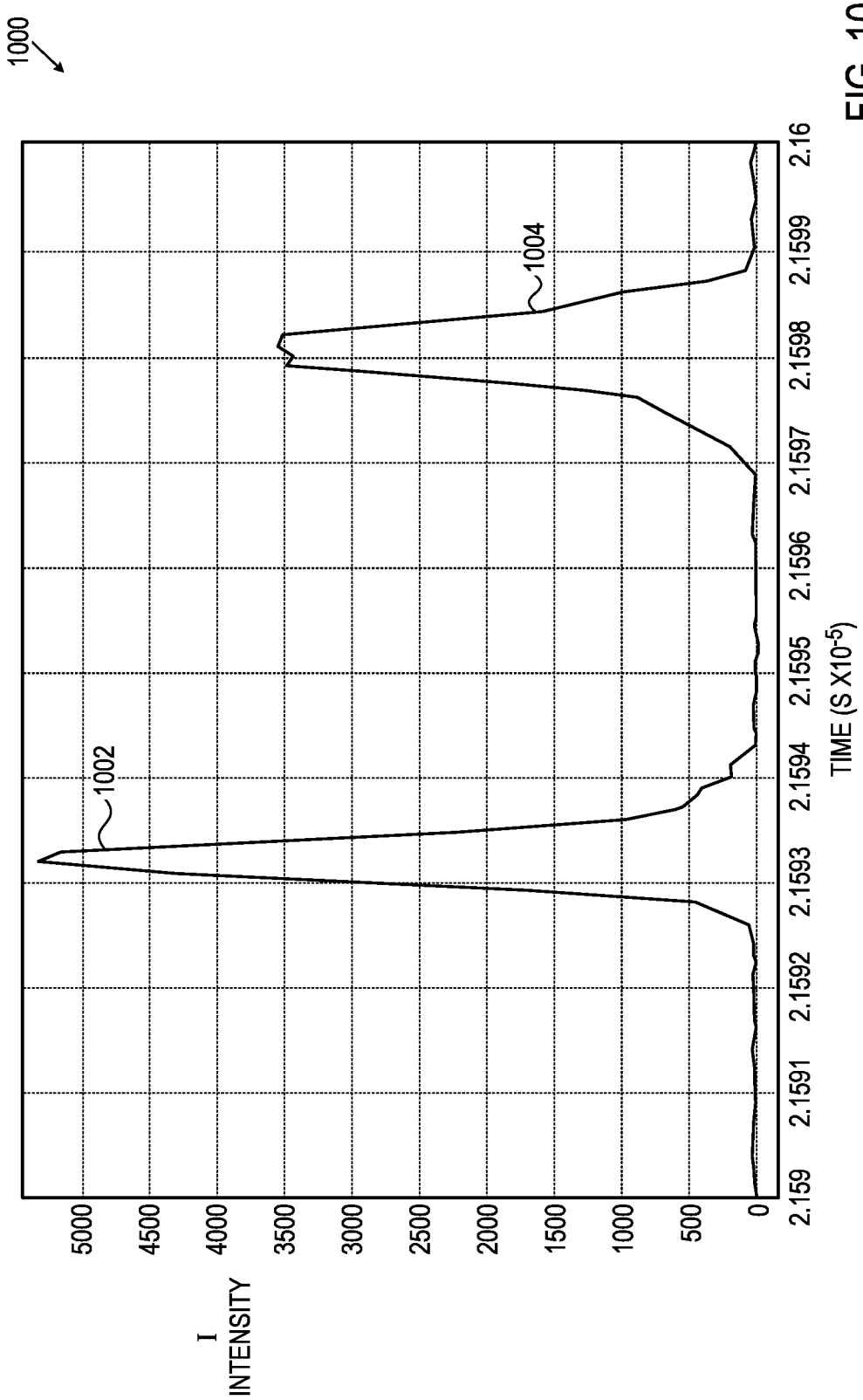
2.1599
2.16
TIME (S X10-5)

FIG. 9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1002
1004
I
INTENSITY
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
2.159
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2.1592
2.1593
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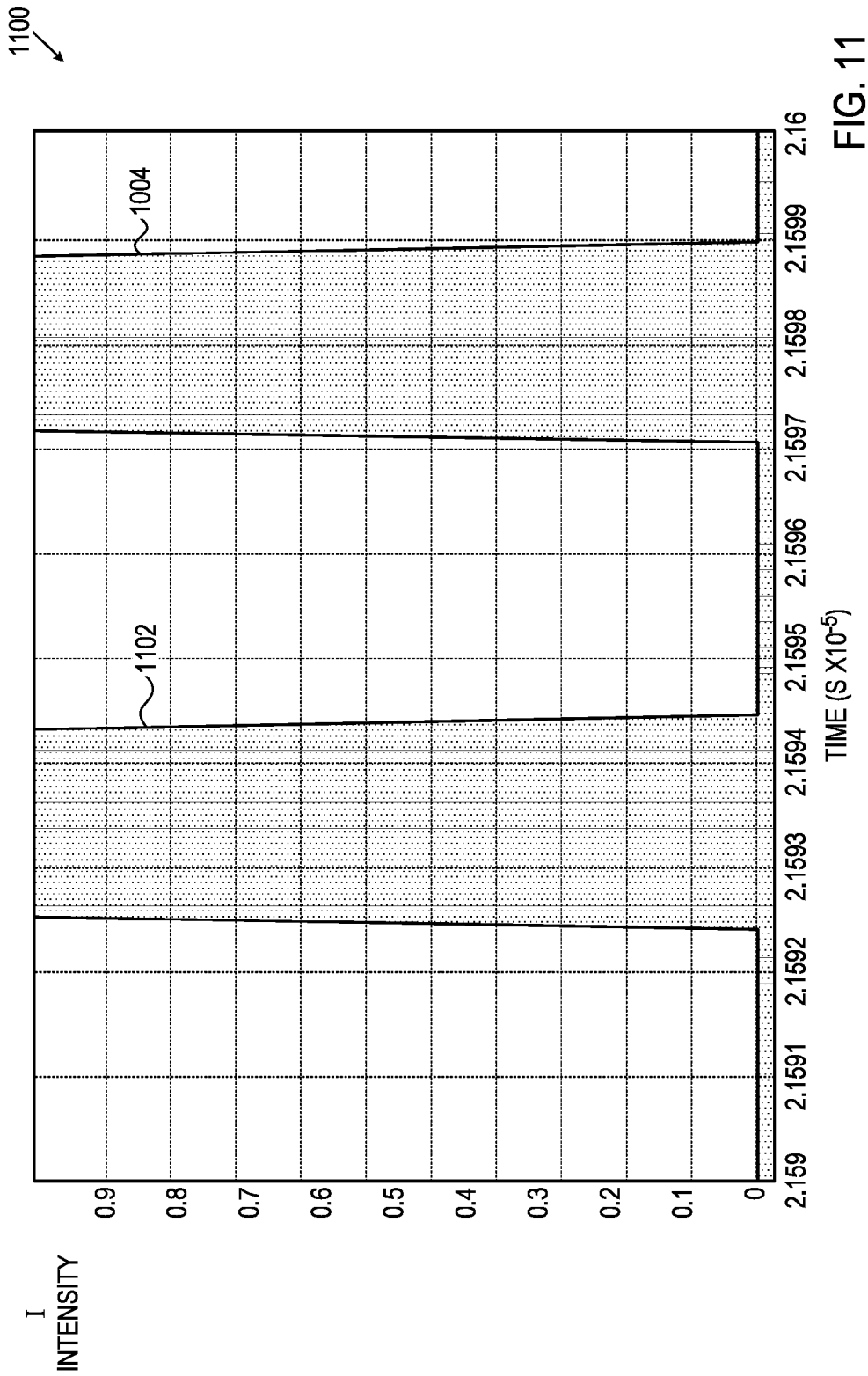
2.1596
2.1597
2.1598
2.1599
2.16
TIME (S X10-5)

FIG. 10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I
INTENSITY
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0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2.159
2.1591
2.1592
2.1593
2.1594
2.1595
2.1596
2.1597
2.1598
2.1599
2.16
TIME (S X10-5)

FIG. 11

KITS, COMPOSITIONS AND METHODS FOR DETECTING A BIOLOGICAL CONDITION

CROSS-REFERENCE

This application is a continuation application of application Ser. No. 14/296,317, now U.S. Pat. No. 8,945,913, filed on Jun. 4, 2014, which is a divisional application of Ser. No. 13/716,246, now abandoned, filed Dec. 17, 2012, which is incorporated herein by reference in its entirety.

The disclosures of the co-pending US Provisional Patent Application to Kasdan, et al, filed on Nov. 17, 2012, and titled "Kits, Compositions and Methods for Detecting a Biological Condition" and the co-pending US Provisional Patent Application to Kasdan, et al, filed on Nov. 17, 2012, and titled "Kits, Compositions and Methods for Rapid Chemical Detection" are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates generally to apparatus and methods for detecting a biological condition, and more specifically to methods and apparatus for detecting a biological condition in small fluid samples.

BACKGROUND OF THE INVENTION

There are numerous medical conditions which are hard to diagnose. Often diagnosis by a physician is based on the physician's observation of combinations of symptoms in a patient. This sometimes leads to misdiagnosis. Furthermore, the patient's response to a treatment, whether drug or other modality is often followed up by physician's observation.

Many laboratory tests are performed in the diagnostic arena on a bodily specimen or fluid to determine a biological condition in a patient. However, these tests are performed off-line in diagnostic laboratories. Often, the laboratory services are only provided during a single 8-hour shift during the day and tend to be labor intensive. Some prior art publications in the field include, inter alia, U.S. Pat. Nos. 8,116,984, 2006215155 and 2012187117.

Despite the inventions mentioned hereinabove, there still remains an unmet need to provide improved apparatus and methods for detecting and diagnosing biological conditions in a patient.

SUMMARY OF THE INVENTION

It is an object of some aspects of the present invention to provide improved apparatus and methods for detecting and diagnosing biological conditions in a patient.

In some embodiments of the present invention, improved methods, apparatus and kits are provided for detecting and diagnosing a biological condition in a patient.

In other embodiments of the present invention, a method and kit is described for providing rapid detection of biological moieties in a sample from a patient.

In further embodiments of the present invention, a method and kit is disclosed for providing detection of biological moieties in a small fluid sample from a patient.

There is thus provided according to an embodiment of the present invention, a kit for evaluating a biological condition in a patient, the kit comprising;

a) a disposable element for receiving a biological specimen and for combining said specimen with at least one composition;

b) at least one composition comprising at least one detector moiety adapted to react with said specimen to form a reaction product, when said patient has said biological condition; and

c) at least one reporter element adapted to provide an indication of reaction product thereby providing the indication of the biological condition. Additionally, according to an embodiment of the present invention, the kit further comprises;

d) instructions for using the kit.

Furthermore, according to an embodiment of the present invention, the disposable element is a disposable cartridge.

Moreover, according to an embodiment of the present invention, the disposable cartridge is a disposable microfluidics cartridge.

Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least one of the following elements:

a) a reservoir;
b) a pump;
c) a valve;
d) a conduit;
e) a motor;
f) a miniaturized flow cell;
g) a transport channel;
h) a microfluidic element;
i) a compressed gas holding element;
j) a compressed gas releasing element;
k) a nozzle element;
l) a mixing element;
m) a bellows element;
n) software adapted to activate said elements according to a specific sequence; and
o) hardware to activate said elements according to a specific sequence.

Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least two of the elements.

Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least three of the elements.

Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least four of the elements.

Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least five of the elements.

Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least ten of the elements.

Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least twenty of the elements.

Additionally, according to an embodiment of the present invention, the disposable microfluidics cartridge comprises at least thirty of the elements.

According to an embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with one hour.

According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with thirty minutes.

According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with fifteen minutes.

According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with ten minutes.

According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with five minutes.

According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with one minute.

According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with thirty seconds.

According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with ten seconds.

According to another embodiment of the present invention, the microfluidics kit is configured to provide the rapid indication with one second.

There is thus provided according to an embodiment of the present invention, a microfluidics assay kit for performing a rapid biological assay, the kit comprising;

a) a disposable element comprising a reactant, the disposable element being adapted to receive a sample comprising a biological entity and for combining said reactant with said biological entity to form a reaction product; and

b) at least one reporter element adapted to provide a rapid indication of disappearance of said reactant thereby providing rapid assay of the biological entity.

There is thus provided according to an embodiment of the present invention, a microfluidics assay kit for performing a rapid assay of a biological entity, the kit comprising;

a) a disposable element comprising a reactant, the disposable element being adapted to receive a sample comprising the biological entity and for combining said reactant with said biological entity to form a reaction product; and

b) at least one reporter element adapted to provide a rapid indication of appearance of said reaction product thereby providing rapid assay of the biological entity. There is thus provided according to an embodiment of the present invention, a composition for evaluating a biological condition, the composition comprising;

a. a sample composition comprising at least one of;

i. a bodily specimen comprising a target moiety;

ii. a positive control moiety; and

iii. a negative control moiety;

b. a detection composition comprising at least one of;

i. at least one target antibody;

ii. at least one positive control identifying antibody; and

iii. at least one negative control identifying detection moiety or characteristic; and

c. at least one reference composition comprising at least one of;

i. a target signal reference composition; and

ii. a reference identifier composition.

There is thus provided according to another embodiment of the present invention a composition for evaluating a biological condition, the composition comprising;

a. a sample composition comprising at least one of;

i. a bodily specimen comprising a target moiety;

ii. a positive control moiety; and

iii. a negative control moiety;

b. an antibody composition comprising at least one of;

i. at least one target antibody (CD64 antibody);

ii. at least one positive control identifying antibody (CD163); and

iii. at least one negative control identifying antibody or characteristic; and

c. at least one reference composition comprising at least one of;

i. a target signal reference composition; and

ii. a reference identifier composition.

Additionally, according to an embodiment of the present invention, the composition further comprises at least one conditioning moiety comprising;

a. at least one lysis reagent; and

b. at least one diluent.

Furthermore, according to an embodiment of the present invention, the biological condition is selected from a group consisting of blood diseases such as leukemia, thrombocytopenia immune system disorders, local infections, urinary tract disorders, autoimmune diseases and sepsis.

Moreover, according to an embodiment of the present invention the bodily specimen is selected from a group consisting of blood, serum, plasma, urine, saliva, cerebrospinal fluid (CSF), serous fluid, peritoneal fluid and synovial fluid.

According to another embodiment of the present invention, the target moiety includes a CD64 surface antigen on neutrophils.

Additionally, according to a further embodiment of the present invention, the positive control moiety includes monocytes and the negative control includes lymphocytes. Additionally, according to an embodiment of the present invention, the target moiety is CD64 on neutrophils, the positive control moiety includes CD64 expression on monocytes, and the negative control moiety includes lymphocytes without CD64 expression.

Further, according to an embodiment of the present invention, the target indicator is bound to a signaling moiety on the at least one target antibody.

Yet further, according to an embodiment of the present invention, the at least one reference composition includes beads.

Additionally, according to an embodiment of the present invention, the beads include polystyrene microbeads.

Moreover, according to an embodiment of the present invention, the target antibody reference composition includes a first fluorescent signal and the reference identifier composition includes a second fluorescent signal.

Furthermore, according to an embodiment of the present invention, the first fluorescent signal includes FITC and the second fluorescent signal includes Starfire Red fluor.

There is thus provided according to an embodiment of the present invention, a method of quantifying a biomarker in a sample, comprising;

a. contacting the sample with a fluorescently-labeled binding moiety that specifically binds to the biomarker;

b. detecting a first fluorescent signal from at least a portion of the labeled sample;

c. detecting a second fluorescent signal from a population of fluorescently-labeled particles, wherein the population includes a known fluorescent intensity over a fixed time; and

d. normalizing the first fluorescent signal to the second fluorescent signal, thereby quantifying the biomarker, wherein the normalizing includes using a device comprising software capable of comparing the first and second fluorescent signal.

Furthermore, according to an embodiment of the present invention, the biomarker is a sepsis biomarker.

Moreover, according to an embodiment of the present invention, the biomarker is CD64 or CD163.

Additionally, according to an embodiment of the present invention, the sample is a blood sample.

According to another embodiment of the present invention, the fluorescent label of the binding moiety and the fluorescent label of the particles is the same fluorescent label.

Further, according to an embodiment of the present invention, the binding moiety is an antibody.

According to an embodiment of the present invention, the software is capable of recognizing a specific lot of fluorescently-labeled particles.

Moreover, according to an embodiment of the present invention, the individual fluorescent signals include at least one first fluorescent signal and at least one second fluorescent signal.

Additionally, according to an embodiment of the present invention the fluorescently-labeled binding moiety targets a first cell population and a second cell population in the sample.

According to another embodiment of the present invention the detection of binding of the binding moiety to the second cell population provides an internal positive control for the sample.

Furthermore, according to an embodiment of the present invention, the binding moiety is anti-CD64 antibody and the first cell population includes polymorphonuclear leukocytes.

Yet further, according to an embodiment of the present invention, the second cell population includes monocytes.

According to an embodiment of the present invention, the method further comprises the step of determining the presence of at least one cell population in the sample that is not bound by the binding moiety, thus providing an internal negative control for the sample.

There is thus provided according to another embodiment of the present invention, a composition for evaluating a biological condition, the composition comprising;

a. a sample comprising at least one of;
   i. a bodily specimen comprising a target moiety;
   ii. a positive control moiety; and
   iii. a negative control moiety;
b. an antibody composition comprising at least one of;
   i. at least one target antibody;
   ii. at least one positive control identifying antibody; and
   iii. at least one negative control identifying antibody or characteristic; and
c. at least one reference composition comprising at least one of;
   i. a target antibody reference composition; and
   ii. a reference identifier composition.

According to an embodiment of the present invention, the composition further comprises at least one conditioning moiety comprising;

a) at least one lysis reagent; and
b) at least one diluent.

There is thus provided according to another embodiment of the present invention, a method of determining the presence or absence of sepsis in a subject, the method including;

a) contacting a blood sample from the subject with a fluorescently-labeled binding moiety specific to a sepsis marker, wherein the volume of the blood sample is 50 μL or smaller; and
b) detecting the presence, absence or level of the binding moiety in the sample, thereby determining the presence or absence of sepsis in the subject.

There is thus provided according to another embodiment of the present invention, a method of quantifying a biomarker in a sample, comprising;

a) contacting the sample with a fluorescently-labeled binding moiety that specifically binds to the biomarker;
b) detecting a first fluorescent signal from at least a portion of the labeled sample;
c) detecting a second fluorescent signal from a population of fluorescently-labeled particles, wherein the population includes a known fluorescent intensity over a fixed time; and
d) normalizing the first fluorescent signal to the second fluorescent signal, thereby quantifying the biomarker, wherein the normalizing includes using a device comprising software capable of comparing the first and second fluorescent signal.

According to some embodiments, the sample may be liquid, according to other embodiments, the sample may be a colloid or suspension. According to further embodiments, the sample may be a solid, such as in a powder or crystal form.

Typical turnaround times for diagnostic prior art assays are 30-120 minutes. Often, the time lost in waiting for laboratory results can lead to a further deterioration in a patient, and sometimes death. In some cases, the physician has to act without having the laboratory results. This can lead to providing the patient with the wrong treatment. The present invention provides rapid assays to save lives and provide fast correct treatments to a patient.

There is thus provided according to an embodiment of the present invention automated method of determining the presence or absence of sepsis in a subject, including;

a) contacting a blood sample from the subject with a fluorescently-labeled binding moiety specific to a sepsis marker, wherein the volume of the blood sample is 50 μL or smaller; and
b) detecting the presence, absence or level of the binding moiety in the sample, thereby determining the presence or absence of sepsis in the subject within twenty minutes.

Additionally, according to an embodiment of the present invention, the sepsis marker is CD64.

Furthermore, according to an embodiment of the present invention, a second sepsis marker is CD163.

Moreover, according to an embodiment of the present invention, the method further includes contacting the blood sample with a second fluorescently-labeled binding moiety specific for a second sepsis marker.

Further, according to an embodiment of the present invention, the sepsis marker is CD64 and the second sepsis marker is CD163.

Additionally, according to an embodiment of the present invention, the binding moiety is an antibody.

Moreover, according to an embodiment of the present invention, the detecting step is performed in a device capable of receiving the sample and capable of detecting the binding moiety.

Additionally, according to an embodiment of the present invention, the method further includes the step of calibrating the device by detecting a population of the fluorescently-labeled particles.

According to another embodiment of the present invention, the particles include the same fluorescent label as the fluorescently-labeled binding moiety.

Additionally, according to an embodiment of the present invention, the method further includes a second population of particles that include the same fluorescent label as the second fluorescently-labeled binding moiety.

Moreover, according to an embodiment of the present invention, the method further includes performing an internal calibration after the detecting the fluorescently-labeled binding moiety.

Notably, according to an embodiment of the present invention, the calibration is completed in less than 5 minutes.

According to some embodiments, the particles are microbeads.

Additionally, according to an embodiment of the present invention, the method is performed in less than 15 minutes.

Furthermore, according to an embodiment of the present invention, the method, further includes the step of determining the presence of at least one cell population in the sample that is not bound by the binding moiety, thus providing an internal negative control for the sample.

The present invention will be more fully understood from the following detailed description of the preferred embodiments thereof, taken together with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in connection with certain preferred embodiments with reference to the following illustrative figures so that it may be more fully understood.

With specific reference now to the figures in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. **1** is a simplified schematic illustration showing an apparatus for detecting a biological condition, in accordance with an embodiment of the present invention;

FIG. **2** is a simplified flow chart of a method for detecting a biological condition, in accordance with an embodiment of the present invention;

FIG. **3** is a simplified schematic illustration showing a methodology for detecting a biological condition associated with a CD64 cell surface antigen, in accordance with an embodiment of the present invention;

FIG. **4** is a simplified flow chart of a method for detecting a biological condition associated with a CD64 cell surface antigen, in accordance with an embodiment of the present invention;

FIG. **5**A is a graphical output of a fluorescent detection assay of a non-activated neutrophil signature associated with the method of FIGS. **3-4**, in accordance with an embodiment of the present invention;

FIG. **5**B is a graphical output of a fluorescent detection assay of an activated neutrophil signature, associated with the method of FIGS. **3-4**, in accordance with an embodiment of the present invention;

FIG. **5**C is a graphical output of a fluorescent detection assay of a monocyte signature, associated with the method of FIGS. **3-4**, in accordance with an embodiment of the present invention;

FIG. **5**D is a graphical output of a fluorescent detection assay of a reference bead signature, associated with the method of FIGS. **3-4**, in accordance with an embodiment of the present invention;

FIG. **6** is a simplified flow chart of a method for differentiating between different particles, in accordance with an embodiment of the present invention;

FIG. **7** is a graphical output of fluorescence from reference beads in eight wavebands, in accordance with an embodiment of the present invention;

FIG. **8** is a graphical output of data from FIG. **7** after a first mathematical manipulation, in accordance with an embodiment of the present invention;

FIG. **9** is a graphical output of data from FIG. **7** after a second mathematical manipulation, in accordance with an embodiment of the present invention;

FIG. **10** is a graphical output of data from FIG. **7** after a third mathematical manipulation, in accordance with an embodiment of the present invention; and

FIG. **11** is a graphical output of an event locator, based on data from FIG. **8-10**, in accordance with an embodiment of the present invention.

In all the figures similar reference numerals identify similar parts.

DETAILED DESCRIPTION OF THE INVENTION

In the detailed description, numerous specific details are set forth in order to provide a thorough understanding of the invention. However, it will be understood by those skilled in the art that these are specific embodiments and that the present invention may be practiced also in different ways that embody the characterizing features of the invention as described and claimed herein.

International patent application publication no. WO2011/128893 to Kasdan et al., describes a device, system and method for rapid determination of a medical condition and is incorporated herein by reference.

The microfluidic cartridges of the present invention may be any suitable cartridge as shown in the figures or any of the prior art cartridges described or cited herein, such as, but not limited to, those described in USD669191 S1, US20120266986 A1, EP1846159 A2, US2012275972, WO11094577A, US2007292941A and EP1263533 B1.

Reference is now made to FIG. **1**, which is a simplified schematic illustration showing an apparatus **100** for detecting a biological condition, in accordance with an embodiment of the present invention.

Apparatus **100** is a kit comprising a cartridge **102** and a number of chemical/biochemical reactants termed herein, treatment compositions. The treatment compositions are adapted to react, at least in part, with biological specimen, such as a body specimen, to be introduced to the apparatus. The body specimen may be a bodily fluid such as, but not limited to, blood, serum, plasma, urine, saliva, cerebrospinal fluid (CSF), serous fluid, peritoneal fluid and synovial fluid. Additionally or alternatively, the body specimen may be a solid such as a hair, a tooth part, a bone part or a piece of cartilage.

Apparatus **100** comprises a specimen receiving element **118**, adapted to transfer the specimen to a sample composition chamber **104**. The sample composition chamber comprises on or more transfer elements **105**, adapted to transfer the specimen from the sample composition chamber to one or more other locations in the cartridge. In the non-limiting example shown in FIG. **1**, transfer element **105** is a conduit in fluid connection with a treatment chamber **112**.

Additionally, the cartridge comprises a number of treatment composition chambers **106**, **108**, **110**, adapted to respectively house a corresponding number of treatment compositions **120**, **122**, **124**. These treatment compositions may be

liquid, solid or combinations thereof. Apparatus **100** is typically sold commercially as a kit with the treatment compositions disposed therein. In some cases, the apparatus **100** may be adapted for a one-off test and may be disposable. In other cases, the apparatus may be re-used. A re-usable apparatus may be adapted to receive additional external compositions (not shown) or may have a plurality of treatment compositions, wherein only a portion is used for each test.

The apparatus may be constructed and configured such that the treatment composition comprises proteins attached to a surface, such as to beads. A plurality of beads or other structural elements with proteins attached to their surfaces can be made by any one or more of the following methodologies:

- simple attachment such as by adsorption via electrostatic or hydrophobic interactions with the surface, entrapment in immobilized polymers, etc.
- non-covalent or physical attachment;
- covalent bonding of the protein to the bead surface
- biological recognition (e. g., biotin/streptavidin).
- requires two steps: a first layer is formed by silane chemistry such that the surface presents a reactive group (e. g.,epoxy, amino, thiol, etc.), and a second layer (e. g., the protein to be immobilized or a linker molecule) is covalently attached via the immobilized reactive groups.
- covalent attachment to functionalized polymer coatings on the interior of the device or linkage to the free end of a self-assembled monolayer (SAM) on a gold surface.

The reaction type may include any one or more of antigen-antibody binding, sandwich (such as antibody-antigen-antibody), physical entrapment, receptor-ligand, enzyme-substrate, protein-protein, aptamers, covalent bonding or biorecognition.

Cartridge **102** further comprises at least one transfer element **107**, **109**, **111** in fluid communication with each respective of treatment composition chamber, each transfer element also being in fluid communication with treatment chamber **112**. These elements are typically microfluidic channels and may be designed for mixing, such as being tortuous in shape.

Various methodologies for transferring the contents of the treatment composition chambers and the sample composition chamber via the transfer elements to the treatment chamber may be employed, some of which are known in microfluidics technologies. These include air blowing, suction, vacuuming, mechanical transfer, pumping and the like.

Cartridge **102** further comprises at least one transfer element **113** in fluid communication with treatment chamber **112** and with an evaluation chamber **114**.

Optionally, evaluation chamber **114** is further in fluid communication with a transfer element **115**, adapted to remove the contents of the evaluation chamber for disposal outside the cartridge. Alternatively, the evaluation chamber may have no external disposal means.

Table 1 shows some representative applications of apparatus **100** and methods of the present invention.

TABLE 1

Applications of the apparatus and methods of this invention.

Application	Type of Test	Relevant Figures in this invention	Typical Prior Art Laboratory Turnaround time (TAT)- see references	This invention Turnaround time (TAT)	References
Application #1 - CD64 Infection & Sepsis	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	U.S. Pat. No. 8,116,984, Davis, BH et al., (2006)
1 - Fetal Hemoglobin Test	Plasma Protein	FIGS. 1-2 and 6-8D	4 hours	10 minutes	Dziegiel et al. (2006)
2 - Low Platelet Count	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Segal, H. C., et al. (2005):
3 - Resolving BLAST Flag for hematology Lab	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Guerti, K., et al.
4 - CD34 Stem Cell Enumeration Assay	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Sutherland et al. (1996)
5 - Platelets Activation Assay CD62	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Graff et al. (2002) Divers, S. G., et al. (2003)
6 - D-dimer (Bead based protein)	Plasma Protein	FIGS. 1-2 and 6-8D	4 hours	10 minutes	Stein et al. (2004) Rylatt, D. B., et al. (1983):
7 - Chorioamnioitis CD64	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Hillier et al. (1988)
8 - CD20 Cell Quantitation (Therapy Monitoring	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Rawstron et al. (2001) Cheson et al. (1996)
9 - CD52 Cell quantitation (Therapy Monitoring)	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Rawstron et al. (2001)
10 - Circulating Tumor Cells	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Cristofanilli et al. (2004
11 - Reticulated Platelet Assay	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Matic et al. (1998) Ault et al (1993) Wang et al. (2002)

TABLE 1-continued

Applications of the apparatus and methods of this invention.

Application	Type of Test	Relevant Figures in this invention	Typical Prior Art Laboratory Turnaround time (TAT)- see references	This invention Turnaround time (TAT)	References
12 - Bacteria Detection in platelet packs			4 hours	10 minutes	Blajchman et al (2005) McDonald et al. (2005)
13 - Platelet Associated Antibodies	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Michelson (1996)
14 - Residual Leukocyte Count in blood products	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Bodensteiner, (2003)
15 - CD4 HIV AIDS	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Rodriguez (2005). Dieye et al. (2005)
16 - Leukemia Panels - Very complex	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Drexler et al (1986)
17 - Bladder Cancer Screening in Urine - Urine sample	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Ramakumar et al (1999) Lotan et al. (2009)
18 - HLA DR Sepsis and Immunosuppression	Surface Marker	FIGS. 1-2 and 3-5D	4 hours	10 minutes	Hershman et al. (2005) Perry et al (2003)
19 - RECAF Protein for Canine and other Cancers	Plasma Protein	FIGS. 1-2 and 6-8D	4 hours	10 minutes	Moro et al. (2005).
20 - CytoImmun - Cervical Screening			4 hours	10 minutes	Hilfrich et al. (2008)
21 - Procalcitonin (Bead Based Protein) + Feasibility	Plasma Protein	FIGS. 1-2 and 6-8D	4 hours	10 minutes	Assicot et al. (1993) Christ-Crain et al. (2004)

Reference is now made to FIG. **2**, which is a simplified flow chart **200** of a method for detecting a biological condition, in accordance with an embodiment of the present invention.

It should be understood that each of the steps of the method may take a predetermined period of time to perform, and in between these steps there may be incubation and/or waiting steps, which are not shown for the sake of simplicity.

In a sample transferring step **202**, a sample, such as a bodily specimen is transferred from outside apparatus **100** via receiving element **118** into sample composition chamber **104** and then to the treatment chamber **112**. According to some embodiments, the volume of the specimen or sample is less than 200 μL, less than 100 μL, less than 50 μL, less than 25 μL or less than 11 μL.

Thereafter, treatment composition **120** is transferred via transfer element **107** to the treatment chamber in a composition transfer step **204**. In some cases, there may be a treatment composition disposed in the treatment chamber.

Depending on the nature of the treatment composition and sample/specimen type, there may be a requirement to mix or agitate the treatment chamber contents in an optional mixing step **206**. This may be performed by using a small stirbar (not shown) disposed in the chamber. Additionally or alternatively, this may be effected by the fluid dynamics of kit. Additionally or alternatively, stirbars may be disposed in any of the other chambers in the apparatus.

Typically, the total sample volumes are in the range of 10 to 1000 μL, 100 to 900 μL, 200 to 800 μL, 300 to 700 μL, 400 to 600 μL, or 420 to 500 μL.

According to some embodiments, the volume of the treatment composition chambers **106**, **108**, **110** (also called blisters) is from about 1 μL to 1000 μL. According to other embodiments, the volume of the specimen is from about 10 μL to 200 μL. According to other embodiments, the volume of the specimen is about 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 μL.

According to some embodiments, the volume of the treatment compositions **120**, **122**, **124** is at most about 500 μL. According to other embodiments, the volume of the specimen is at most about 200 μL. According to other embodiments, the volume of the specimen at most about 500, 450, 400, 350, 300, 250, 200, 180, 160, 140, 120, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, or 1 μL.

According to some embodiments, the volume of a reactant is at least about 1 μL. According to other embodiments, the volume of the specimen is from about 10 μL. According to other embodiments, the volume of the specimen is at least about 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 μL.

The sequence of transfer of the various treatment compositions may be important to the reaction sequence and is typically predefined. Steps **204**-**206** may be performed, for example on treatment composition chamber **106**, thereafter on treatment composition chamber **108** and thereafter on treatment composition chamber **110**. In some cases, some of these steps may be performed concurrently.

In a checking step **208**, it is ascertained whether all the compositions required for the sample treatment have been transferred to the treatment chamber. If any compositions remain, then steps **204**-**206** are performed on the subsequent treatment composition chamber(s). If no further treatment

compositions require transfer, then the sample/specimen is transferred from chamber **104** into the treatment chamber.

Thereafter, in a second sample transfer step **210**, the sample is transferred from the sample composition chamber into the treatment chamber.

According to some embodiments, step **210** may be performed before steps **204-208**. If required, an optional mixing step **212** to the contents of the treatment chamber may be performed.

In a transferring step **214**, the contents of the treatment chamber are transferred to the evaluation chamber.

The evaluation chamber **114** is configured and constructed for one or more evaluation steps **216**. These may include any of the following, or combinations thereof:

a) transfer of radiation there-through,
b) impinging radiation thereupon;
c) detecting reflected, refracted, and/or transmitted radiation,
d) detecting emitted radiation;
e) capturing one or more images thereof;
f) performing image analysis on the captured images;
g) measuring electrical characteristics of the treated specimen;
h) impinging sonic energy thereon;
i) detecting sonic energy therefrom; and
j) analyzing the outputs of any one or more of the above steps.

According to some embodiments, the cartridge is introduced into a system as described in International patent application publication no. WO2011/128893 to Kasdan et al., incorporated herein by reference.

The results of the evaluation step are then outputted in a results outputting step **218**.

According to some embodiments; the apparatus may have on-board means for showing a result, such as a colorimetric strip (not shown). Additionally or alternatively, the results are displayed in a display unit, separate and remote from apparatus **100**.

Reference is now made to FIG. **3**, which is a simplified schematic illustration showing a methodology **300** for detecting a biological condition associated with a CD64 cell surface antigen, in accordance with an embodiment of the present invention.

According to some embodiments, the method is carried out in the apparatus shown in FIG. **1** and as described herein. A biological specimen, such as a blood sample, is aspirated via specimen receiving element **118** to sample composition chamber **104**, and then to treatment chamber **112**. The sample is typically of a volume in the range of 10-200 μL.

The blood sample is typically whole blood recently removed from a patient. The whole blood comprises mainly red blood cells (also called RBCs or erythrocytes), platelets and white blood cells (also called leukocytes), including lymphocytes and neutrophils. Increased number of neutrophils, especially activated neutrophils are normally found in the blood stream during the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure and some cancers.

A cocktail **304** comprising antibodies to CD64 and antibodies to CD163 is introduced to the treatment chamber (see Davis et al. (2006)). Each antibody type is typically tagged by a specific fluorescent tag.

The contents of the chamber are incubated and/or mixed as is required to bind the activated blood neutrophils with the CD64 tagged antibody (also called a marker) to form activated neutrophils with CD64 marker **310**, and/or monocyte with a CD64 tagged antibody and a CD**163** tagged antibody **312**. Lymphocytes with no markers **314** are present in the contents, as well as unaffected RBCs **316**.

Thereafter, a lysis reagent or diluent **306** is introduced into treatment chamber **112**. In the case of a lysis reagent, it is adapted to lyse red blood cells to form lysed red blood cells **324**. Additionally, reference/calibration beads **308** are added to the treatment chamber. These are used to calibrate the outputs, as is explained with reference to FIGS. **5**A-**5**D hereinbelow.

CD64 (Cluster of Differentiation **64**) is a type of integral membrane glycoprotein known as an Fc receptor that binds monomeric IgG-type antibodies with high affinity. Neutrophil CD64 expression quantification provides improved diagnostic detection of infection/sepsis compared with the standard diagnostic tests used in current medical practice.

CD163 (Cluster of Differentiation **163**) is a human protein encoded by the CD163 gene. It has also been shown to mark cells of monocyte/macrophage lineage.

Reference is now made to FIG. **4**, which is a simplified flow chart **400** of a method for detecting a biological condition associated with a CD64 cell surface antigen, in accordance with an embodiment of the present invention.

According to some embodiments, the method is carried out in the apparatus shown in FIG. **1** and as described herein. In a first transferring step **402**, a biological specimen, such as a blood sample is aspirated via specimen receiving element **118** to sample composition chamber **104**. The sample is typically of a volume in the range of 10-200 μL.

Typically, the total sample volumes are in the range of 10 to 1000 μL, 100 to 900 μL, 200 to 800 μL, 300 to 700 μL, 400 to 600 μL, or 420 to 500 μL.

According to some embodiments, the volume of the treatment composition chambers **106**, **108**, **110** (also called blisters) is from about 1 μL to 1000 μL. According to other embodiments, the volume of the specimen is from about 10 μL to 200 μL. According to other embodiments, the volume of the specimen is about 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 μL.

According to some embodiments, the volume of the treatment compositions **120**, **122**, **124** is at most about 500 μL. According to other embodiments, the volume of the specimen is at most about 200 μL. According to other embodiments, the volume of the specimen at most about 500, 450, 400, 350, 300, 250, 200, 180, 160, 140, 120, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, or 1 μL.

According to some embodiments, the volume of a reactant is at least about 1 μL. According to other embodiments, the volume of the specimen is from about 10 μL. According to other embodiments, the volume of the specimen is at least about 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, or 500 μL.

In an addition step **404**, a cocktail of tagged antibodies to CD64 and to CD163 is added to the treatment chamber **112** and is incubated with the blood sample. In the incubation phase of this step, the antibodies bind activated neutrophils with CD64 marker **310**, and/or monocytes activated with a CD64 tagged antibody and a CD163 tagged antibody **312**.

In a lysis reagent addition step **406**, the lysis reagent is added to the treatment chamber and thereby lyses at least some of the RBCs in the chamber.

At any suitable time, typically following lysis step **406**, reference beads are added to the contents of the treatment chamber in a reference bead adding step **408**.

After a predefined period of time, an analysis step **410** is performed to analyze the fluorescent emission signatures from the contents. This is described in further detail with reference to FIGS. **5**A-**5**D. According to some examples, the

evaluation chamber **114** is constructed and configured to allow cells to pass through a reading zone **130** such that each cell passing therethrough is analyzed individually. The assay sensitivity is around 86% and its specificity is around 87% (Hoffmann, 2011).

The time required to complete an assay using apparatus **100** of the present invention varies depending on a number of factors, with non-limiting examples that include described herein. In some embodiments, the time required to complete an assay is from about 0.5 to 100 minutes. In other embodiments, the time required to complete an assay is from about 1 to 20 minutes. In still other embodiments, the time required to complete an assay is from about 1 to 10 minutes. In some examples, the time required to complete an assay is from about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 50, 60, 80, or 100 minutes.

Reference is now made to FIG. **5**A, which is a graphical output of a fluorescent detection assay of a non-activated neutrophil signature **500** associated with the method of FIGS. **3-4**, in accordance with an embodiment of the present invention. The non-activated tagged neutrophils each emit a signal **502** at wavelength W**1** of an intensity I**1**. The wavelengths shown in FIGS. **5**A-**5**D represent a peak wavelength of waveband outputs detected, as are shown in FIGS. **7-11**.

FIG. **5**B shows a graphical output of a fluorescent detection assay of an activated neutrophil signature **510**, associated with the method of FIGS. **3-4**, in accordance with an embodiment of the present invention. Each activated tagged neutrophil emits an activated neutrophil signature **512** at wavelength W**1** of an intensity I**2**. Typically I**2** is greater than I**1**. In some cases the difference in signatures **512** and **510** may be detected by an image analysis, a fluorescent emission radiation count or by other qualitative or quantitative methods known in the art. The current example is not meant to be limiting.

Turning to FIG. **5**C, there can be seen a graphical output of a fluorescent detection assay of a monocyte signature **520**, associated with the method of FIGS. **3-4**, in accordance with an embodiment of the present invention. The monocyte signature comprises a first signal **522** at a first wavelength W**1** of an intensity I**3** and a second signal **524** at a second wavelength W**2** of an intensity I**4**.

FIG. **5**D shows a graphical output of a fluorescent detection assay of a reference bead signature **530**, associated with the method of FIGS. **3-4**, in accordance with an embodiment of the present invention. The reference bead signature comprises a first signal **532** at a first wavelength W**1** of an intensity I**1** (similar or equal to non-activated tagged neutrophils' signal **502**) and a second signal **534** at a second wavelength W**3** of an intensity I**5**.

This methodology enables the identification and quantification of activated neutrophils by intensity of signature **512** of the CD64 tag. Monocytes are identified by the double signal signature **522**, **524**, acting as a positive control. Reference beads are identified by the unique signal **534** at wavelength W**3**. The intensity of signal **532** at wavelength W**1** provides a reference level of CD64 for the comparison of intensity of 512 of the neutrophils.

Lymphocytes with no markers **330** (FIG. **3**) act as a negative control and should provide no fluor signature, but may be detected by their scattering or other characteristics. Further details of some embodiment of this assay procedure are described in U.S. Pat. No. 8,116,984 and in Davis, B H et al., (2006).

Reference is now made to FIG. **6**, which is a simplified flow chart of a method **600** for differentiating between different particles, in accordance with an embodiment of the present invention.

The input to the processing is a time series from each of the channels in the eight channel photomultiplier array **601**. In addition, data from multiple scatter channels **609** is introduced. Each fluorescent time series and scatter time series may be processed individually employing respective spectral crosscorrelation algorithm **606** and scatter algorithm **607** to smooth it and minimize noise. Two possible processing methods are boxcar averaging algorithm **602** and matched filtering algorithm **604**. In addition, groups of individual channels may be correlated to yield a multiple spectral crosscorrelations **606**. One or more of these derived time series may be used to determine event locations.

Once an event is located in the eight channel time series the composition of that event in terms of known fluorophore signatures is determined using a minimum mean square error fit **610**. The event is now described in terms of its composition of known fluors. Each event thus described is stored in an event store, i.e. memory, together with the data from the eight time series for that event and its description **612**. Based on the fluor composition for each event in the data store, it is possible to determine the type of particle. For example, a neutrophil **616** is characterized by the single fluor attached to the CD64 antibody shown in FIG. **5** as W**1**. Thus events that are preponderantly characterized by the single fluor attached to the CD64 antibody are identified as neutrophils.

Similarly, monocytes **618** are characterized by fluors W**1** and W**2** so that an event with both of these fluor signatures is identified as a monocyte. Similarly, a bead **620** is characterized by an event that has fluors W**1** and W**3**. Lymphocytes **622** do not express significant fluorescence but are identified by their scatter as events. Events that do not match any of the known combinations of the fluorophores are identified as rejects **626**.

Given the population of identified events, the median intensity of the neutrophil population and the median intensity of the bead population are determined. The ratio of the neutrophil median to the bead median is the desired Leuko**64** index. The positive control value is determined as the median intensity of the CD64 fluorophore bound to monocytes divided by the median intensity of the same fluorophore on the bead population. The negative control value is determined by the median intensity of the CD64 fluorophore bound to lymphocytes. These are the key steps in performing the Leuko**64** assay.

FIG. **7** is a graphical output **700** of fluorescence from reference beads in eight wavebands, in accordance with an embodiment of the present invention. This figure shows the smoothed signals from the eight channel PMT array for two reference beads. The amplitude for each waveband is shown on the same graph. The corresponding wavelength range is shown for each plot **702**, **706**, **708**, **710**, **712**, **714**, **716**, **718** in the legend box. The two fluorophores signatures present in this plot are **702**,**706** and **708** for FITC, which is the fluorophore attached to the CD64 antibody and **710**, **712** for Starfire Red, which is the fluorophore identifying the reference beads.

Reference is now made to FIG. **8**, which is a graphical output **800** of data from FIG. **7** after a first mathematical manipulation, in accordance with an embodiment of the present invention. FIG. **8** shows the cross correlation of wave bands one two and three corresponding to wavelength 500 to 525, 525 to 550, and 552 to 575 nm. This cross correlation is computed by multiplying the boxcar smoothed time series

corresponding to these wavelengths. This signal will have a high-value when an event containing the FITC fluorophore is present.

FIG. **9** is a graphical output **900** of data from FIG. **7** after a second mathematical manipulation, in accordance with an embodiment of the present invention. FIG. **9** shows the cross correlation of wave bands **3**, **4** and **5** corresponding to wavelengths 550 to 575, 575 to 600, and 600 to 625 nm. This signal will have a high-value when an event containing the PE fluorophore is present.

FIG. **10** is a graphical output **1000** of data from FIG. **7** after a third mathematical manipulation, in accordance with an embodiment of the present invention. FIG. **10** shows the cross correlation of wave bands **7** and **8** corresponding to wavelengths 650 to 675, and 675 to 700 nm. This signal will have a high-value when an event containing the Starfire Red fluorophore is present.

FIG. **11** is a graphical output **1100** of an event locator, based on data from FIG. **8-10**, in accordance with an embodiment of the present invention. FIG. **11** shows the event locations determined from the cross correlations computed in FIGS. **8**, **9** and **10**. The solid fill area **1102** corresponds to the region where any of the cross correlations **802**, **902** and **1002** exceeded a predefined threshold. Similarly, the solid fill area **1104** corresponds to the region where any of the cross correlations **804**, **904** and **1004** exceeded a predefined threshold. This then completes the event location process.

EXAMPLE

Application No. 1-CD64 Infection & Sepsis

A cartridge **102** (FIG. **1**) is prepared for receiving a blood sample. The cartridge comprises a number of treatment composition chambers **106**, **108**, **110**, adapted to respectively house a corresponding number of treatment compositions **120**, **122**, **124**. These compositions are described in further detail in U.S. Pat. No. 8,116,984 and in Davis, B H et al., (2006)), incorporated herein by reference. In brief, Reagent A comprises a mixture of murine monoclonal antibodies (contains buffered saline), Reagent B-10× Concentrated Trillium Lyse solution (contains ammonium chloride), Reagent C-suspension of 5.2 μm polystyrene beads labeled with Starfire Red and fluorescein isothiocyanate (FITC), (contains <0.1% sodium azide and 0.01% Tween 20).

In a sample transferring step **202** (FIG. **2**), a 10 uL blood sample, is transferred from outside apparatus **100** via receiving element **118** into sample composition chamber **104** and then on to treatment chamber **112** in a transferring step **214**.

An antibody composition (Reagent A) **120** comprising CD64 antibodies is transferred via transfer element **107** to the treatment chamber **112** in a composition transfer step **204**.

These two steps combined with mixing step **206** take around four minutes using cartridge **102** of the present invention.

A lysis buffer (Reagent B) **122** is also added and mixed with the resultant mixed composition. This step and mixing all the compositions takes around three minutes using cartridge **102** of the present invention. Reference beads (Reagent C) **308** are added to the treatment chamber.

The evaluation chamber **114** is configured and constructed for one or more evaluation steps **216**.

According to some embodiments, the cartridge is introduced into a system as described in International patent application publication no. WO2011/128893 to Kasdan et al., incorporated herein by reference. This system has software associated therewith for computing the CD64 and CD163 indices on leukocytes.

The results of the evaluation step are then outputted in a results outputting step **218**. According to this example, the time taken from the introduction of the small blood sample to obtaining an indication of sepsis is less than 15 minutes, typically around 10 minutes.

From a user point of view, the following steps are performed:

1) The user adds drop of blood to the cartridge **102** and seals it. (10 μL are metered out by microfluidics).
2) Blister A (**106**) is pressed, releasing 100 μL of Reagent A. Mixing in the cartridge is controlled by the cartridge handling unit (CHU), followed by a 4-minutes incubation.
3) Blister B (**108**) is pressed, releasing ~250 μL of Reagent B. Mixing in the cartridge is controlled by the CHU, followed by a 3-5-minutes incubation.
4) Magnetic stirbar is activated, stirring the bead suspension (Reagent C).
5) Blister C (**110**) is pressed, releasing 100 μL of Reagent C. Mixing in the cartridge is controlled by the CHU. According to one example, Reagent A is a mixture of murine monoclonal antibodies-diluted 1:5 in buffered saline (PBS+0.5% BSA); Reagent B is a Trillium Lyse solution (at working concentration); Reagent C is a suspension of 5.2 μm polystyrene beads labeled with Starfire Red and FITC, diluted 1:100 in PBS+0.01% Tween 20.
6) The sample is read by the optoelectronics core, and collected to the reading below.
7) Data is analyzed automatically and result is presented.
8) The cartridge is disposed as biohazard.

TABLE 2

Comparison of Prior art methodology with the methodology of the present invention for detecting sepsis using CD64 and CD163 antibodies. LeukoDx device- present invention

Step	Description	Volume (uL)	Duration (min)	comments
1	Mixing blood and antibodies	Blood- 10 Abs- 50	4	
2	Adding RBC lysis buffer	250	3	Might require heating the buffer to 37 C.
3	Incubating, Vortexing		3	
4	Adding normalization beads	2	Less than 1	
5	Reading		Less than 1	
	Total	312	10	

In the case of sepsis, by "normalization" is meant taking the ratio of the median of the target population fluorescence emission to the median of the reference bead population fluorescence emission.

According to some embodiments, the readout may comprise an optoelectronics core, which enables identification and detection of fluorescent signals.

The CCD in the core, used for focusing, can also be used to read chemiluminescent signals. The readout to user may also indicate where the result falls relative to reference ranges.

The contents of these publications are incorporated by reference herein where appropriate for teachings of additional or alternative details, features and/or technical background.

It is to be understood that the invention is not limited in its application to the details set forth in the description contained herein or illustrated in the drawings. The invention is capable of other embodiments and of being practiced and carried out in various ways. Those skilled in the art will readily appreciate that various modifications and changes can be applied to the embodiments of the invention as hereinbefore described without departing from its scope, defined in and by the appended claims.

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What is claimed is:

1. A test cartridge for assaying for possible infection or sepsis in a subject, comprising a single use microfluidic cartridge that is adapted to receive a blood sample from a subject, the microfluidic cartridge comprising:

a) a sample composition chamber adapted for receiving a blood sample from a subject;

b) a first pre-filled microfluidic blister comprising an antibody mixture comprising fluorescently tagged CD64 and fluorescently tagged CD163 antibodies;

c) a second pre-filled microfluidic blister comprising a cell lysis reagent;

d) a third pre-filled microfluidic blister comprising fluorescently tagged beads comprising two fluorescent tags, wherein at least one of the two fluorescent tags is different than the fluorescently tagged CD64 and fluorescently tagged CD163 antibodies;

d) a treatment compartment adapted for fluid mixing, wherein the treatment compartment is in fluid communication with the sample composition chamber, the first pre-filled microfluidic blister, the second pre-filled microfluidic blister, and the third pre-filled microfluidic blister;

e) a pump connected to the treatment compartment; and

f) an evaluation chamber fluidly connected to the treatment chamber and comprising a reading zone.

2. A Cartridge Handling Unit (CHU) for detection of possible infection or sepsis in a subject, the CHU adapted to receive a test cartridge and pre-programmed to perform at least the following steps:

a) pressing a first blister of the test cartridge thereby releasing an antibody mixture and permitting it to contact with a blood sample, wherein the antibody mixture comprises fluorescently tagged CD64 and fluorescently tagged CD163 antibodies;

b) allowing the blood sample and the antibody mixture to contact for a predetermined time thereby fluorescently tagging blood cells in the blood sample;

c) pressing a second blister of the test cartridge thereby releasing fluorescently tagged beads comprising two fluorophores to contact the blood sample and antibody mixture, wherein at least one of the two fluorescent tags is different than the fluorescently tagged CD64 and fluorescently tagged CD163 antibodies;

d) individually flowing tagged blood cells and tagged beads through a reading zone;

e) exciting the fluorescent tags of the beads and of the antibodies;

f) measuring fluorescent signals of the fluorescently tagged blood cells and of the fluorescently tagged beads simultaneously; and

g) using the measuring to provide an indication of the possibility of infection or sepsis in the subject.

3. The CHU of claims **2**, wherein the test cartridge further comprises a pump or an air blowing element.

4. The CHU of claims **3**, wherein the contact between the bloods sample and the antibody mixture is facilitated using the pump or the air blowing element.

5. The microfluidic cartridge of claims **1**, wherein the pump is a bellow pump.

6. The microfluidic cartridge of claim **1**, wherein the microfluidic cartridge is valveless.

7. The microfluidic cartridge of claim **1**, wherein a volume of any of the blisters is from about 1 microliter to 1000 microliters.

8. The microfluidic cartridge of claim **1**, wherein a volume of the blood sample is about 10 microliters.

9. The CHU of claim **2**, wherein the CHU is pre-programmed to perform the steps in less than 15 minutes.

10. The microfluidic cartridge of claim **1**, wherein the microfluidic cartridge further comprises a tortuous shaped channel.

11. The microfluidic cartridge of claim **1**, wherein the fluorescently tagged beads comprise Starfire Red.

12. The microfluidic cartridge of claim **1**, wherein the blood sample is whole blood.

13. The microfluidic cartridge of claim **1**, wherein the blood sample comprises erythrocytes or leukocytes, and wherein the leukocytes comprise lymphocytes or neutrophils.

14. The microfluidic cartridge of claim **1**, wherein the blood sample comprises leukocytes.

15. The CHU of claim **2**, wherein the CHU is pre-programmed to press a third blister of the test cartridge thereby releasing a cell lysis reagent and permitting the cell lysis reagent to contact the blood sample and the antibody mixture.

16. The CHU of claim **15**, wherein pressing the third blister occurs after pressing the first blister and before pressing the second blister.

17. The microfluidic cartridge of claim **1**, wherein the cell lysis reagent comprises ammonium chloride.

18. The microfluidic cartridge of claim **1**, wherein the antibodies are murine monoclonal antibodies.

19. The CHU of claim **2**, wherein using the measuring to provide an indication comprises employing at least one of cross-correlation algorithm, boxcar averaging algorithm, filtering algorithm or minimum mean square error fit.

20. The CHU of claim **2**, wherein the CHU is pre-programmed to determine a type of the blood cells.

21. The CHU of claim **2**, wherein the test cartridge is valveless.

22. The CHU of claim **2**, wherein a volume of any of the blisters is from about 1 microliter to 1000 microliters.

23. The CHU of claim **2**, wherein a volume of the blood sample is about 10 microliters.

24. The CHU of claim **2**, wherein the test cartridge comprises a tortuous shaped channel.

25. The CHU of claim **2**, wherein the fluorescently tagged beads comprise Starfire Red.

26. The CHU of claim **15**, wherein the cell lysis reagent comprises ammonium chloride.

27. The CHU of claim **2**, wherein the antibodies are murine monoclonal antibodies.

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